PB85-144111

Effects of Copper, Nickel and Zinc on the Life Cycle of the Caddisfly 'Clistoronia magnifica' (Limnephilidae)

(U.S.) Corvallis Environmental Research Lab., OR

1984

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	PB85-144111
EPA-600/J-84-185		

4. TITLE AND SUBTITLE
(Journal Version)
Effects of Copper, Nickel and Zinc on the Life Cycle of the Caddisfly Clistoronia magnifica (Limnephilidae)

5. REPORT DATE
1984

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Corvallis, Oregon 97333

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/02

15. SUPPLEMENTARY NOTES
Environmental Toxicology and Chemistry 3:645-649, 1984 (Oct-Dec)

16. ABSTRACT

The caddisfly Clistoronia magnifica (Banks)(Limnephilidae) was exposed to copper, nickel and zinc in life cycle tests. The copper test included continuous exposure of first generation aquatic larvae and pupae, second generation larvae and pupae and third generation larvae. The aerial adults and eggs were exposed only to body burdens obtained as larvae and pupae. Nickel and zinc tests included first and second generation exposure. Copper concentrations of ≥17 µg/L prevented completion of the life cycle, and significant reduction in adult emergence occurred at 13.0 µg/L. The no-observed-effect level for copper was 8.3 µg/L. Nickel concentrations of ≥250 µg/L prevented completion of the life cycle. The no-observed-effect level for nickel was 66 µg/L. At the highest zinc concentration (5,243 µg/L), no effects were observed on any of the life stages.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	6
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

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U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-84-185
JOURNAL ARTICLE

Environmental Toxicology and Chemistry, Vol. 3, pp. 645–649, 1984
Printed in the USA. Pergamon Press Ltd.

1984 SETAC

Short Communication

EFFECTS OF COPPER, NICKEL AND ZINC ON THE LIFE CYCLE OF THE CADDISFLY *CLISTORONIA MAGNIFICA* (LIMNEPHILIDAE)

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(*Received* 13 *October* 1983: *Accepted* 5 *March* 1984)

Abstract — The caddisfly *Clistoronia magnifica* (Banks) (Limnephilidae) was exposed to copper, nickel and zinc in life cycle tests. The copper test included continuous exposure of first-generation aquatic larvae and pupae, second-generation larvae and pupae and third-generation larvae. The aerial adults and eggs were exposed only to body burdens obtained as larvae and pupae. Nickel and zinc tests included first and second-generation exposure. Copper concentrations of ≥17 μg/L prevented completion of the life cycle, and significant reduction in adult emergence occurred at 13.0 μg/L. The no-observed-effect level for copper was 8.3 μg/L. Nickel concentrations of ≥250 μg/L prevented completion of the life cycle. The no-observed-effect level for nickel was 66 μg/L. At the highest zinc concentration (5,243 μg/L), no effects were observed on any of the life stages.

Keywords — Caddisfly *Clistoronia magnifica* Copper Nickel Zinc Life cycle test

INTRODUCTION

Copper, nickel and zinc are common water pollutants found in a variety of industrial and mining discharges. Copper, as copper sulfate, is also applied to aquatic environments to control algae or as a component of some pesticides. An extensive literature review and summary of laboratory acute and chronic toxicity tests can be found in the U.S. Environmental Protection Agency Water Quality Criteria Documents for copper [1], nickel [2] and zinc [3]. As can be seen in these reviews, there are few long-term chronic tests with aquatic insects and none with caddisflies.

The test species for this study, *Clistoronia magnifica* (Limnephilidae), is representative of a large group of aquatic insects, the caddisflies (Trichoptera), which are important links in aquatic food chains and serve as primary food sources for many fish species [4]. *C. magnifica* has been successfully reared through several life cycles in the laboratory by Anderson [5,6] and at our laboratory, and has been used in one chronic life cycle test [7].

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The purpose of this study was to determine the effect of copper, nickel and zinc on the survival, adult emergence and reproduction of *C. magnifica*, and to determine the no-observed-effect level (the highest concentration with results not significantly different from controls) of each heavy metal for the caddisfly under the stated test conditions.

METHODS AND MATERIALS

Water for all flow-through tests was obtained from a well and was aerated vigorously prior to use. Water temperatures ranged between 14.5°C at night and 16°C in the afternoon. Total hardness, alkalinity, pH and dissolved oxygen were monitored (Table 1) using procedures described by the American Public Health Association [8].

Stock solutions were prepared separately for each metal by dissolving reagent-grade copper chloride, nickel chloride and zinc chloride salts in acidified (0.1 ml HNO_3/L) well water. The solution was pumped from a 400-liter polyethylene stock tank to a diluter [7]. Water samples for total metal analyses were taken from test containers weekly and analyzed on a Perkin-Elmer 305b atomic absorption spectrophotometer with direct aspiration or furnace techniques [8,9]. Mean measured concentrations, with standard deviations, for total copper and nickel are given in Tables 2 and 3. Copper levels in larval, pupal and adult tissues were also measured using the methods of Adrian [10,11].

The test species, *C. magnifica*, was obtained from cultures established by Dr. N. H. Anderson, Oregon State University, Corvallis, OR [5]. The species inhabits submerged marginal vegetation and open sediments in lakes of the Cascade Mountains in Oregon [6]. We provided our cultures with quartz sand as case material, alder and maple leaves for case materials and food, and wheat and frozen brine shrimp as additional food [12]. Adults fed on honey droplets placed on the top of the flight cage.

A continuous-flow dilutor system [7], capable of delivering 10 concentrations of metal to test containers, was used for chronic testing. Copper, nickel or zinc stock solution was pumped into the incoming dilution water in the first chamber of the dilutor at a constant delivery rate of 37 ml/min. Metal solutions were serially diluted (0.7 dilution ratio) and delivered through glass lines to 10-liter test containers at a flow rate of 100 ml/min.

Glass, slate-bottomed 10-liter aquaria were used as test containers. Fine-mesh stainless steel wire screen was provided in each aquarium for use by pupae (pharate adults) to climb out of the water prior to emergence. A nylon mesh flight cage [7] was placed over each aquarium to retain adults. All aquaria were cleaned and debris siphoned out as needed. A photoperiod of 14 h light:10 h dark was provided for all tests, using wide-spectrum fluorescent lamps. The light intensity was about 11 ft-c at the water surface.

Tests were started with larvae, and data were recorded through pupation, adult emergence, mating, egg deposition, egg hatch and development of young second- and third-generation larvae. Tests were started by randomly distributing the same

Table 1. No-observed-effect levels and water quality values for *C. magnifica* life cycle tests with copper, nickel and zinc

Test	No-observed-effect level [μg/L][a]	Water temp. (°C)	pH range	Mean (± SD) total hardness (mg/L $CaCO_3$)	Mean (± SD) total alkalinity (mg/L $CaCO_3$)
Copper	8.3	15 ± 2	7.2 - 7.4	26 ± 1	26 ± 1
Nickel	66	15 ± 2	6.8 - 7.4	54 ± 8	45 ± 7
Zinc	>5,243	15 ± 2	6.6 - 7.3	31 ± 5	39 ± 12

[a]The highest concentration with results not significantly different ($\alpha = 0.05$) from controls.

Table 2. Effects of chronic exposure to copper on the life cycle of the caddisfly *C. magnifica*

Mean (±SD) measured copper concn. (μg/L)	First generation		Second generation	
	Percent[a] larval survival	Percent adult emergence	No. of larvae found	No.[b] of adults
98 ± 17	60	0	0	0
71 ± 13	40	0	0	0
51 ± 10	80	0	0	0
36 ± 4	100	0	0	0
25 ± 3	80	30[c]	0	0
17 ± 3	90	30[c]	0	0
13 ± 3	70	30[c]	130	17
8.3 ± 3	80	60	80	6
5.2 ± 2	80	50	125	16
4.2 ± 2	100	80	132	23
Control	80	70	84	17
Control	80	80	146	13

[a]Ten larvae exposed at each concentration (only five larvae at 98, 71, 51 μg/L and second control).
[b]Eggs and larvae produced (third generation) in concentrations of ≤13 μg/L.
[c]Significantly different from controls ($\alpha = 0.05$).

number of larvae, usually 10, into each aquarium. Because of the limited number of animals available for testing, only five larvae were placed in those high metal concentrations in which all were expected to die. Some larvae, pupae and adults were weighed and retained for tissue copper analyses.

The life cycle test conducted with copper chloride was started with fifth-instar larvae, and the second generation was exposed until adults emerged, mated, deposited eggs and larvae developed (8-month test). The test with nickel chloride was started with late third- and early fourth-instar larvae, and the test conducted with zinc chloride was started with third-instar larvae.

Chi-square analysis of the chronic data was used to determine which treatments were significantly different from controls.

RESULTS AND CONCLUSIONS

Copper

The no-observed-effect level of copper (Table1) was 8.3 μg/L. At 13.0 μg/L, copper caused significant reduction in adult emergence. Concentrations of ≥17 μg/L prevented completion of the life cycle. Sixty percent of the larvae at the highest concentration tested (98 μg/L) survived to pupation (i.e., sealed the ends of their cases); 40% survived pupation to emerge as swimming pupae

Table 3. Effects of chronic exposure to nickel on the life cycle of the caddisfly *C. magnifica*

Mean (±SD) measured nickel concn. (μg/L)	First generation		Second generation
	Percent[a] larval survival	Percent adult emergence	Maximum no. of larvae observed
3,669 ± 243	50	0	0
1,669 ± 151	80	0	0
690 ± 77	100	0	0
250 ± 14	100	50[b]	0
144 ± 21	100	40[b]	11
66 ± 7	100	80	244
Control	90	90	86

[a]Ten larvae exposed at each concentration.
[b]Significantly different from controls ($\alpha = 0.05$).

(Table 2). Pupation occurred at all concentrations, but no adults emerged at concentrations of ≧36 μg/L. Successful adult emergence occurred at 25, 17 and 13 μg/L, but emergence was significantly lower than in the controls. Time from emergence of adults in the controls until appearance of eggs was about 15 d at 15°C.

The appearance of the swimming pupa (pharate adult) was followed, usually within 1 h, by emergence of adults, which moved up into the flight cage. Not all adults exposed to copper were normally formed: Crumpled or incompletely inflated and flattened wings, malformed legs, and incomplete detachment of the pupal skin occurred at higher copper concentrations. At high concentrations, some pupae emerged from the case but adults were unable to successfully emerge from the pupal skin. Adults emerged at 25 and 17 μg/L copper, but mating was not observed; mating was observed at concentrations of ≦13 μg/L. Extruded but unfertilized egg masses were observed at 25 and 17 μg/L, often at the death of the adult. Normal egg masses containing viable eggs and second-generation larvae were observed at concentrations of ≦13 μg/L copper. About 13 d were required for eggs to hatch and for the young larvae to migrate away from the egg mass.

The life cycle of the second generation was completed successfully and eggs were produced and larvae developed at concentrations of ≦13 μg/L copper. Development from hatching to adult emergence during the second generation took about 115 d at 15°C. Time from second-generation adult emergence to production of third-generation eggs took from 11 to 25 d. Even though there was a significant effect on first-generation adult emergence at 13 μg/L, the second-generation larvae and adults apparently were not affected. Life cycle parameters at the lower copper concentrations (5.2 and 4.2 μg/L) were similar to those of the controls. Tissue concentrations of copper were generally found to be directly proportional to water concentrations. A larva exposed to 25 μg/L Cu for 30 d had the highest tissue level (249 μg/g Cu). The lowest tissue concentration (12.5 μg/g Cu), was found in larvae from controls.

Nickel

The no-observed-effect level of nickel (Table1) was 66 μg/L and concentrations of ≧690 μg/L prevented completion of the life cycle. Adult emergence occurred at 250 μg/L nickel (Table 3) but was significantly less than that of the controls; no deformed adults were observed. Half (5 of 10) of the caddisflies at the highest nickel concentration of 3,669 μg/L died before pupation, and the two larvae that did survive never pupated, indicating a possible blocking of normal development. This also occurred with one caddis larva at 1,669 μg/L. Mating, egg production and some second-generation larvae were observed at 144 μg/L. Greater numbers of second-generation larvae were found at 66 μg/L. Development of second-generation larvae stopped at the first instar (head capsule width of 0.32 mm) at all nickel concentrations, whereas some control larvae developed to the third instar before the test was terminated. Time from emergence of adults until the appearance of eggs in the control aquaria was about 18 d at 15°C (Table 3). About 20 d elapsed before the eggs hatched and young larvae began to feed. Developmental times were delayed in the animals exposed to nickel, when compared to the controls.

Zinc

No effects of zinc were observed on any life stage of the caddisfly at any of the test concentrations (≦5,243 μg/L); consequently, a no-observed-effect level concentration could not be determined (Table1). Ninety percent survival of larvae occurred at 5,243 μg/L with 70% emergence of swimming pupae from the pupal case. Sixty percent adult emergence occurred after 76 d of exposure at 5,243 μg/L zinc.

Acute tests were conducted as part of this study to obtain 96-h LC_{50} values to compare

with the chronic values. However, we were unable to kill 50% of the animals at 22 mg/L copper, 37 mg/L nickel or 61 mg/L zinc. The discrepancy between 96-h LC_{50} values and chronic test values, illustrated by the relative acute insensitivity of *C. magnifica* to heavy metals and sensitivity to chronic exposure, indicates the need for long-term bioassays to predict potential damage to aquatic species.

Acknowledgement — We wish to thank Dr. N. Anderson, Oregon State University, for supplying caddisflies and for assistance in establishing our own laboratory colony. We also thank W. Miller, A. Purcell, A. Sipols, R. Titus, K. Wickman and C. Wise for assistance with metal and water chemical analyses, and A. Stinchfield and B. Williams for aid during testing.

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